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PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

EXAMINER	
KIM, YOUNG J	
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte
JEN-I MAO, SHUJUN LUO, ALAN EWING, DAVID H. LLOYD, and STEPHEN C. MACEVICZ

Appeal 2008-2260
Application 09/867,201
Technology Center 1600

Decided: November 18, 2008

Before DONALD E. ADAMS, LORA M. GREEN, and FRANCISCO C. PRATS, *Administrative Patent Judges*.

GREEN, *Administrative Patent Judge.*

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the Examiner's final rejection of claims 3 and 9-14.[1] We have jurisdiction under 35 U.S.C. § 6(b).

STATEMENT OF THE CASE

The claims are directed to a method of nucleotide sequencing. Claims 3 and 9 are representative of the claims on appeal, and read as follows:

3. A method of simultaneously determining a signature sequence for each polynucleotide in a population of polynucleotides, the method comprising the steps of:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates, such that substantially every different polynucleotide has a different oligonucleotide tag attached;

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated;

(c) separating the polynucleotide fragments into size classes,

wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease;

(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment;

(e) copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class; and

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[1] Claims 1, 2, and 4-8 were cancelled by an amendment filed with the Appeal Brief, and claims 15, 16 stand withdrawn from consideration (App. Br. 2).

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports.

9. A method of simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates, wherein substantially every different polynucleotide has a different tag, the method comprising the steps of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes,

wherein said step of generating includes: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides;

separating the size classes of polynucleotide fragments, wherein said separating includes forming substantially homogeneous populations of each of said size classes of said mixture by physical separation;

amplifying and labeling the tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of each such polynucleotide fragment;

separately hybridizing the labeled tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached to each of a plurality of microarrays, each microarray of the plurality having the same spatially addressable hybridization sites; and

determining each signature sequence in the sample by a set of signals generated at hybridization sites having the same address on each of the plurality of microarrays.

The Examiner relies on the following references:

Brenner	US 5,763,175	Jun. 9, 1998
Wong	US 5,935,793	Aug. 10, 1999
Strathmann	US 6,480,791 B1	Nov. 12, 2002

We affirm.

ISSUE (Obviousness)

The Examiner concludes that claims 3 and 9-14 are rendered obvious by the combination of Brenner and Wong.

Appellants contend that the combination of Brenner and Wong does not render obvious a method of sequencing wherein a size ladder of polynucleotide fragments is generated for each tag-polynucleotide conjugate.

Thus, the issue on Appeal is: Whether the combination of Brenner and Wong renders obvious a method of sequencing wherein a size ladder of polynucleotide fragments is generated for each tag-polynucleotide conjugate, wherein the size ladder is generated by shortening the polynucleotides of said tag-polynucleotide conjugates (claim 3) or by extending the initializing oligonucleotide by ligating extension oligonucleotides (claim 9)?

FINDINGS OF FACT

FF1 The Examiner rejected claims 3, 9-12 and 14[2] under 35 U.S.C. § 103(a) as being obvious over the combination of Brenner and Wong (Ans. 3).

FF2 Appellants argue independent claim 3 as a separate group from claim 9 and the claims dependent thereon (App. Br. 9-12). Thus, claims 10-12 and 14 stand or fall with claim 9. 37 C.F.R. § 41.37(c)(1)(vii).

FF3 As to claim 3, Appellants only argue steps (b) and (c), and thus waive any argument that the combination fails to teach and/or suggest the remaining steps (*see* App. Br. 9-11).[3]

FF4 As to claim 9, Appellants only argue the limitations of:

> generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments, to form a mixture of said size classes,
>
> wherein said step of generating includes: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides.

(*See* App. Br. 11-14). Thus, Appellants waive any argument that the combination fails to teach and/or suggest the remaining steps.

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[2] The Answer refers to claims 1-12 and 14 in the statement of the rejection (Ans. 3), but acknowledges that claims 1, 2, and 4-8 were canceled (*id.* at 2).

[3] In this decision we consider only those arguments actually made by Appellants. Arguments that Appellants could have made but chose not to make in the Briefs have not been considered and are deemed to be waived. *See* 37 C.F.R. § 41.37(c)(1)(vii).

FF5 Brenner "provides a method of sequencing large numbers of polynucleotides in parallel by using oligonucleotide tags to shuttle sequence information obtained in 'bulk' or solution phase biochemical processes to discrete spatially addressable sites on a solid phase. Signals generated at the spatially addressable sites convey the sequence information carried by the oligonucleotide tag." (Brenner, col. 4, l. 66-col. 5, l. 5.)

FF6 The Examiner finds that "Brenner teaches each and every limitation of the claims rejected under 35 U.S.C. 103 except using shortening to create a size ladder to form size classes." (Ans. 10.)

FF7 As to step (b) of claim 3, the Examiner finds that Brenner's teaching that "'beads are then transferred to reaction mixtures containing Apa I, which cleaves all strands not containing methyl groups, i.e. all the strands that have been selectively amplified," (*id.* at 11 (quoting Brenner col. 20, ll. 60-63) requires that the "PCR amplified products will inherently and necessarily be cleaved into different size polynucleotide fragments by ApaI since ApaI cleaves at every CCCGGG site which is not methylated. Since the PCR products as noted by Brenner, are not methylated, ApaI will cleave at the ApaI site in the primer and any ApaI site naturally present in the cDNAs, which should randomly occur about every 4096 bases." (Ans. 11.)

FF8 As to step (c) of claim 3, the Examiner finds that Brenner's teaching of "'[a]fter, or concurrently with, the 32 PCRs, Bbv I is used to shorten the cDNA inserts of the library," (*id* (quoting Brenner col. 21, ll. 29-32)), as well as the teaching of that the "'amplicon is then cleaved with Bbv I and the S primer segment is removed with magnetic beads coated with avidin" (Ans.

11-12 (quoting Brenner col. 21, ll. 42-45)), "will generate polynucleotide fragments with the same tag with a Type IIs restriction enzyme" (Ans. 12).

FF9 The Examiner finds that "[w]hile Brenner cleaves with ApaI, which will inherently form size classes, Brenner does not teach applying the use of the BbvI shortening enzyme to form the size classes and then separating the resultant polynucleotides into size classes." (*Id.* at 13.)

FF10 As to claim 9, the Examiner relies on the following teaching of Brenner:

> After amplification, the amplicon is methylated to protect internal Bbv I sites, its 3' ends are stripped using T4 DNA polymerase, and dGTP, after which the recessed strands are filled in by the addition of dTTP and dCTP. The amplicon is then cleaved with Bbv I and the S primer segment is removed with magnetic beads coated with avidin. The following adaptor mixture containing a new S primer binding site is then ligated to the T primer segment.

(Ans. 15-16 (quoting Brenner, col. 21, ll. 38-45).)

FF11 According to the Examiner, the passage represents "a precise teaching of the method required by claim 9," as claim 9 requires that the primer first be extended, and as taught by Brenner, the primer is extended in an amplification reaction, with the "T primer segment" serving as the extension oligonucleotide (Ans. 16).

FF12 The Examiner finds further that "Wong expressly teaches a method analogous to that of Brenner, in which size classes [i.e., size ladders] are formed." (*Id.* at 13.) Specifically, the Examiner cites claim 1 of Wong, which recites the steps of "'c) separating said sequencing fragments on the basis of fragment length under conditions effective to resolve fragments

differing in length by a single base, to produce a plurality of resolved size-separated fragments, (d) collecting the size-separated fragments in separate aliquots.'" (*Id.*) According to the Examiner, "Wong then amplifies (ie copies) the fragments and applies them to an array." (*Id.*)

FF13 Specifically, Wong is drawn to "a method of sequencing in parallel a plurality of polynucleotide sample fragments. In the method, a plurality of sample polynucleotide fragments is used to form a mixture of different-length sequencing fragments." (Wong, col. 3, ll. 39-43.) "The sequencing fragments are . . . separated on the basis of size under conditions effective to resolve fragments differing in length by a single base, to produce a plurality of resolved, size-separated bands." (Col. 3, ll. 51-54.) Thus, Wong teaches the concept of generating a size ladder and separating the size classes of polynucleotide fragments.

FF14 Wong teaches that:

> Conveniently, the sample or samples contain polynucleotide fragments within a selected size-range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing. Fragments having selected size ranges may be prepared by standard methods, such as sonication, separation, digestion with endonucleases and exonucleases, chemical degradation, and the like. The size range may be controlled further by subjecting the sample to agarose or polyacrylamide gel electrophoresis, size-exclusion chromatography, or other separation methods, and selecting subfractions having the desired size range.

(Wong, col. 12, ll. 6-16.)

FF15 The Examiner concludes:

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to

> modify Brenner to incorporate the size separation as taught by Wong since Wong teaches "Conveniently, the sample or samples contain polynucleotide fragments within a selected size range, e.g., 400-2000 nucleotides, to achieve a desired sampling frequency for effective shotgun sequencing (see column 12, lines 6-16)."

(Ans. 7.)

PRINCIPLES OF LAW

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) secondary considerations of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). The Supreme Court has recently emphasized that "the [obviousness] analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007). "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *Id.* at 1739. Moreover, an "[e]xpress suggestion to substitute one equivalent for another need not be present to render such substitution obvious." *In re Fout*, 675 F.2d 297, 301 (CCPA 1982).

ANALYSIS

With respect to claim 3, Appellants argue that Brenner does not teach the steps of:

> (b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated;
>
> (c) separating the polynucleotide fragments into size classes,
>
> wherein said steps of generating and separating include forming a plurality of aliquots of tag-polynucleotide conjugates, and *shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot* such that said polynucleotides in different aliquots are shortened a different amount, and wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease.

(App. Br. 9-10 (emphasis in original).)

Brenner teaches, Appellants assert, that the position of the BbvI (the type II restriction endonuclease) site "is constant with respect to the primer binding region. Consequently, the enzyme will cleave at the same position in the target polynucleotide for each primer, thus shortening each target polynucleotide by the same amount." (App. Br. 10 (emphasis removed).) Thus, Appellants argue, the process of Brenner "does not constitute '*shortening by a different amount* said polynucleotides of said tag-polynucleotide conjugates,'" as required by claim 3 (*id.* at 11 (emphasis in original)).

According to Appellants, Wong also does not teach

> forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that

> said polynucleotides in different aliquots are shortened a different amount, wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease.

(*Id.*) Thus, Appellants assert, because "**neither Wong nor Brenner** teaches or suggests this feature of independent claim 3, the claim cannot be found unpatentable over these references." (*Id.*)

Appellants' arguments are not convincing. First, Appellants do not address, however, the Examiner's argument that it is the ApaI that performs the shortening, not the BbV I, and that restriction of the amplicons with the ApaI would inherently form a size ladder (FF7). It would have been well within the level of skill in the art, however, to adapt the method to use any known restriction enzyme for shortening, such as a type II endonuclease (*see* Brenner, col. 15, ll. 10-36 for a discussion of type II endonucleases). As noted by the Court in *KSR*, "[a] person of ordinary skill is also a person of ordinary creativity, not an automaton." 127 S. Ct. at 1742.

Thus, we find that the Examiner has shown by a preponderance of the evidence that Brenner inherently performs shortening of the polynucleotides of said tag-polynucleotide conjugates through the use of an enzyme. Wong then provides motivation to take advantage of the different sizes, by teaching that samples that contain polynucleotide fragments within a selected size range achieves a desired sampling frequency, allowing for effective shotgun sequencing (FF14).

As to claim 9, Appellants argue that claim 9 does not require the use of type IIs enzymes for shortening, but instead includes the limitation:

> *generating a size ladder* for every tag-polynucleotide conjugate such that each size ladder has a plurality of size

> classes of polynucleotide fragments, to form a mixture of said size classes,
>
> *wherein said generating comprises: extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide, and then extending the initializing oligonucleotide by ligating extension oligonucleotides.*

(App. Br. 12 (emphasis in original).)

Claim 9, Appellants assert, "recites 'ligating extension oligonucleotides', in the plural," creating "'a set of polynucleotide fragments . . . each differing in length from one another by integral multiples of the length of the extension oligonucleotide' (i.e. a 'size ladder')." (*Id.* at 12.) Brenner, Appellants argue, "teaches the ligation of a single, same-length adaptor onto each cleaved polynucleotide target (column 21, lines 38-45, as cited by the Examiner), which would not generate a 'size ladder' of polynuclotide fragments as recited in the claim." (*Id.* at 13 (emphasis in original).) Appellants argue further that Wong also does not teach the above cited step of generating a size ladder, and as "**neither Wong nor Brenner** teaches or suggests this feature" claims 9-14 are not unpatentable over their combination (*id.*).

Again, Appellants' arguments are not convincing. First, Appellants have not pointed out why the Examiner is incorrect in finding that T primer segment corresponds to the extension oligonucleotide of Brenner (FF10-11). Second, as noted above as to claim 3, Wong provides the motivation for exploiting the different sizes to achieve effective shotgun sequencing.

Appellants argue further that the motivation provided by the Examiner to combine the references is "misdirected." (App. Br. 13.) According to

Appellants, the Examiner asserts it would have been obvious "to modify Brenner by 'using the size selection of Wong', to 'achieve a sampling frequency . . . useful for shotgun sequencing.'" (App. Br. 13-14 (quoting Final Action, pp. 11-12).)

According to Appellants:

> The "sampling frequencies" [as used in Wong and Brenner] . . . refer to entirely different concepts. Thus, the "size selection of Wong" (i.e. polynucleotide fragments 400-2000 nucleotides in length) has no relevance to "achieving a sampling frequency" as taught in Brenner. The process of "sampling" in Brenner refers to selecting a sample of tag-polynucleotide conjugates from a larger population of tag-polynucleotide conjugates, and is not concerned with the length of the polynucleotides.

(*Id.* at 14.)

We agree with the Examiner, however, that the passage of Wong

> is drawn to sequencing of end regions. This passage refers to a desire to have different size targets in the analysis. Wong then provides a variety of different size targets expressly in the Wong method, Wong also notes "If only one label type is used for detection, sequencing fragments may be processed together (i.e., separated by size .. (see column 17, lines 52-55)." There can be no more direct statement by Wong that different size fragments can be used in the analysis method.

(Ans. 17.)

Finally, the Examiner also rejects claim 13 over the combination of Brenner and Wong as further combined with Strathmann (Ans. 8). In response, Appellants rely on their arguments made with respect to claim 9, thus, claim 13 also falls with claim 9.

Thus, we conclude that the Examiner has set forth a prima facie case that has not been rebutted by Appellants that claims 3 and 9-14 are obvious over the cited prior art, and the rejections on appeal are affirmed.

CONCLUSIONS OF LAW

Thus, we conclude that the combination of Brenner and Wong renders obvious a method of sequencing wherein a size ladder of polynucleotide fragments is generated for each tag-polynucleotide conjugate, wherein the size ladder is generated by shortening the polynucleotides of said tag-polynucleotide conjugates (claim 3) or by extending the initializing oligonucleotide by ligating extension oligonucleotides (claim 9).

TIME LIMITS

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv)(2006).

AFFIRMED

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PERKINS COIE LLP
P.O. BOX 1208
SEATTLE WA 98111-1208